American Journal of Medical Genetics 64:563–567 (1996)

Phenotypic Variability in Monozygotic Twins With Neurofibromatosis 2

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Mutations in the neurofibromatosis 2 (*NF2*) tumor suppressor gene on chromosome 22q12 cause a clinically variable autosomal dominant syndrome characterized by bilateral vestibular schwannomas (VSs), other nervous system tumors, and early onset lenticular cataracts. We studied three pairs of monozygotic (MZ) twins with NF2, all with bilateral VSs, to separate genetic from nongenetic causes of clinical variability. The evaluation included gadolinium-enhanced high-resolution magnetic resonance imaging of the head and spine, neuro-ophthalmic examination with slit lamp, physical examination, and zygosity testing with microsatellite markers. Each MZ pair was concordant for general phenotypic subtype (mild or severe) and often for the affected organ systems. However, the MZ pairs were discordant for some features of disease presentation or progression. For example, all three pairs were discordant for presence or type of associated cranial tumors. We hypothesize that phenotypic differences between NF2 MZ twins are at least partly due to stochastic processes, such as the loss of the second *NF2* allele or alleles of other genes.

KEY WORDS: neurofibromatosis 2; genes, neurofibromatosis 2; genes, suppressor, tumor; twins; phenotype

INTRODUCTION

Neurofibromatosis 2 (NF2) is an autosomal dominant syndrome characterized by bilateral vestibular schwannomas (VSs), other nervous system tumors, and ocular abnormalities such as early onset posterior subcapsular cataracts (PSCC) [Parry et al., 1994]. NF2 occurs in ~1 in 40,000 live births, with half of the cases representing new mutations [Evans et al., 1992a]. The *NF2* tumor suppressor gene has been identified on chromosome 22q12 [Rouleau et al., 1992; Trofatter et al., 1992]. Recently, the diagnostic criteria for NF2 were expanded for first-degree relatives of NF2 patients [CDP, 1994]. Evans et al. [1992b] and Parry et al. [1994] also have suggested that the NF2 diagnostic criteria include sporadic cases with a unilateral VS and either multiple meningiomas or meningioma(s) and two other manifestations of NF2.

The prevalence of clinical manifestations in NF2 increases with age [Parry et al., 1994], and there is marked clinical variability [Evans et al., 1992a,b; Parry et al., 1994]. The mild and severe phenotypes can be defined by age-of-onset ($\geq$ vs. $<$20 years), number of associated intracranial tumors ($<$ vs. $\leq$2), and rare spinal tumors (absent vs. present) [Parry et al., 1994]. Genotype-phenotype correlations in NF2 are probably complex. The mild phenotype may be associated with mutations that preserve the sequence integrity of the C-terminal end of the NF2 protein [Merel et al., 1995]. However, deletions that include the entire *NF2* gene can result in a mild phenotype [Sanson et al., 1993; Watson et al., 1993], and patients with the same mutation can have different phenotypes [Bourn et al., 1994b; Kluwe et al., 1995; Scoles et al., 1995]. Phenotype is similar in some multigeneration NF2 families [Evans et al., 1992a,b; Parry et al., 1994; Sainio et al., 1995], but differs within others [Wertelecki et al., 1988; Baser et al., 1995].

Genetically identical monozygotic (MZ) twins provide an opportunity to differentiate genetic from nongenetic causes of phenotypic variability. A single affected has been reported in dizygotic NF2 twin pairs [Kanter et al., 1980; Kaiser-Kupfer et al., 1989; Parry et al., 1991], but no dually affected twins with NF2 have been reported since the initial description of the disease in 1822 [Wishart, 1822], and relatively few MZ twins have been reported in any tumor suppressor gene syndrome [Liu, 1990; Easton et al., 1993]. In this report, we describe 3 NF2 MZ twin pairs.

Received for publication August 9, 1995; revision received January 25, 1996.

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MATERIALS AND METHODS

The twins were ascertained from the House Ear Clinic (Los Angeles); Childrens Hospital Los Angeles; and the University of Iowa Hospitals and Clinics (Iowa City). Informed consent was obtained from all participants or their parents. The clinical evaluation included gad-olinium-enhanced, high-resolution magnetic resonance imaging (GE-MRI) of the head and (in most cases) full spine, neuro-ophthalmic examination with slit lamp, and physical examination. We defined phenotypic concordance in terms of general phenotypic subtype (mild or severe), but also examined specific disease features.

DNA was obtained from peripheral lymphocytes for five of the six twins, and from paraffin-embedded tissue for the sixth (deceased). Zygosity testing was performed using nine microsatellite markers on different chromosomes: D6S305 [Weissenbach et al., 1993], TH (chromosome 11) [Polymeropoulos et al., 1991a], D12S84 [Weissenbach et al., 1993], CYP19 (chromosome 15) [Polymeropoulos et al., 1991b], D16S403 [Weissenbach et al., 1993], D18S57 [Weissenbach et al., 1993], D19S177 (Human Gene Mapping 11, 1991), D21S215 (sequence obtained from Genome Data Base), and D22S430 [Sainz et al., 1993]. The marker heterozygosities were 0.83, 0.78, 0.92, 0.91, 0.85, 0.86, 0.79 (calculated), 0.65, and 0.68, respectively. Seven markers (excluding D12S84 and D22S430) were used for pair 2.

RESULTS

Each twin pair was identical for all alleles. The histories, summarized in Table I, are described below.

Pair 1 were the third birth to a mother with NF2, who presented with bilateral hearing loss and dizziness at age 66 (after twin 1A was diagnosed). She had bilateral VSs (the larger, on the left, 1.5 cm). At age 64, visual acuity was 20/100 in the left eye and 20/300 in the right eye, with bilateral mixed cataracts (nuclear, posterior subcapsular, and cortical).

Twin A reported a gradual onset hearing loss in the left ear and intermittent tinnitus in the right ear at age 35 and difficulty walking at age 36. She was diagnosed at age 37 with bilateral VSs (left, 0.8 cm; right, 2.0 × 1.5 cm). There were ~12 tumors in the cervical, thoracic, and lumbar spines. Visual acuity was 20/30 in each eye and there was a PSCC in the right eye.

Twin B was asymptomatic prior to her twin's diagnosis, at which time she was imaged and diagnosed with a 1.0 × 0.5 cm left VS and a cranial meningioma. Two years later, she developed a right VS and three tumors of the lumbar spine nerve sheath. She had one large (2.2 cm × 1.0 cm) and two small café-au-lait (CAL) spots on her back and legs. Visual acuity was 20/25 in the left eye and 20/30 in the right eye, with bilateral PSCC and bilateral elevated discs at the nasal border.

Another affected sibling (5 years older) had visual loss at age 38 and was found to have severe bilateral PSCC that reduced corrected visual acuity to 20/200 in the left eye and 20/70 in the right eye. At age 42, he experienced hearing loss and dizziness and was diagnosed at age 43 with bilateral VSs (left, 1.0 cm × 0.7 cm; right, 0.5 cm). The full spine GE-MRI was normal. There was a cutaneous tumor on the right lower leg.

Pair 2 were the only offspring of a clinically unaffected mother (father's affected status unknown). Four months prior to examination at age 11, twin A presented with difficulty walking and running, tingling in his left foot, then progressive pain and weakness in his legs. He was diagnosed with ~6 cervical spine tumors (the thoracic and lumbar spines were not imaged). The spinal tumors included a large (2.8 cm × 2.5 cm × 2.8 cm) extramedullary tumor in the C1-2 and anterior lower medullary region that compressed the medulla and proximal spinal cord, and multiple lesions at C3-7, including a 1.5 cm intramedullary mass at C5. Following operations to remove the extramedullary tumor, he developed quadriparesis and renal failure of unknown etiology. At age 13, he had bilateral hearing loss and was diagnosed with bilateral VSs (left, 2.0 cm × 2.0 cm × 2.0 cm; right, 5.0 cm × 4.0 cm × 4.0 cm) that compressed the medulla and 4th ventricle. There were numerous (>10) CAL spots and cutaneous tumors of the scalp, the retroauricular region adjacent to the external auditory canal, the left ankle, and the midposterior midline spine area. At age 15, he developed bilateral visual loss. Visual acuity was 20/400 in the left eye and unrecordable in the right eye. In the left eye, there was a 0.9 cm subconjunctival mass and nodular lesions of the upper lid. Cataracts were not present. There was severe bilateral papilledema and subretinal exudates with subretinal detachment. In the right eye, there was marked afferent defect associated with probable central retinal vein occlusion. He died at age 17 after progressive neurological deterioration and renal failure.

Twin B was asymptomatic at age 11 but was imaged when his twin was diagnosed. He had a small cervical spine tumor but remained asymptomatic until age 16, when he experienced progressive hearing loss and imbalance for 6 months prior to re-evaluation. GE-MRI evaluation at age 17 revealed bilateral VSs (left, 2.0 cm; right, 3.5 cm × 2.9 cm × 2.7 cm compressing the brainstem). There were multiple tumors throughout the cervical spine and a syrinx at C2-3. The thoracic and lumbar spines were not imaged. There was a 0.8 cm × 1.0 cm mass superior to the optic chiasm (possible optic nerve sheath meningioma) and masses in the anterior right tongue and right masseter muscle. There was also enhancement of the left 5th and 9th–11th cranial nerves consistent with schwannomas. There were numerous (>10) cutaneous tumors on the side, back, forehead, hands, and tip of tongue, but no CAL spots. He had a cataract in the right eye and strabismus diagnosed at age 1 due to a divergent squint. At age 17, visual acuity was 20/30 in the left eye and 20/400 in the right eye. He had nystagmus and a convergent squint. There was a retinal hamartoma, elevated disc, epiretinal membrane formation, and a 6th nerve palsy in the left eye. In the right eye, there were mixed lens opacities (cortical, PSCC, and embryonal) and a subconjunctival mass.

Pair 3 were the only offspring of clinically unaffected parents. Twin A presented at age 6 months with a macular hamartoma in the left eye, a PSCC in the right eye, and nystagmus. Visual acuity was 20/40 in the left eye

TABLE I. Clinical Findings in 3 Pairs of Monozygotic Twins With Neurofibromatosis 2*

Clinical findings	Twin A	Twin B
Pair 1		
1. First symptom	Hearing loss and tinnitus age 35, imbalance age 36	Asymptomatic when imaged at age 37
2. CNS tumors		
VSs	Bilateral	Bilateral
Other cranial	None	Cranial meningioma (1)
Spinal	Cervical, thoracic, and lumbar tumors (~12)	Lumbar tumors (3)
3. Ocular		
Left eye	Visual acuity 20/30 Normal	Visual acuity 20/25 PSCC
Right eye	Visual acuity 20/30 PSCC	Visual acuity 20/30 PSCC
4. Cutaneous	Normal	CAL spots (3)
Pair 2		
1. First symptom	Imbalance, tingling, pain in legs age 10	Hearing loss and imbalance age 16
2. CNS tumors		
VSs	Bilateral	Bilateral
Other cranial	None	Mass superior to optic chiasm, 5th and 9th–11th cranial nerve masses
Spinal	Cervical tumors (~6)	Multiple cervical tumors
3. Ocular		
Left eye	Visual acuity 20/400 Subconjunctival mass and nodular lesions of upper lid, papilledema and exudative retinal detachment	Visual acuity 20/30 Retinal hamartoma, elevated disc, epiretinal membrane formation, 6th nerve palsy
Right eye	Visual acuity unrecordable No cataracts Papilledema and exudative retinal detachment, afferent defect	Visual acuity 20/400 Mixed cataracts (cortical, PSCC, embryonal) Subconjunctival mass
4. Cutaneous	Numerous (>10) skin tumors and CAL spots	Numerous skin tumors, no CAL spots
Pair 3		
1. First symptom	Decreased hearing age 12	Asymptomatic when imaged at age 13
2. CNS tumors		
VSs	Bilateral	Bilateral
Other cranial	Left optic nerve sheath mass, 4th and 9th–12th cranial nerve masses	None
Spinal	Cervical and thoracic tumors (3)	Cervical tumors (3)
3. Ocular		
Left eye	Visual acuity 20/40 Macular hamartoma	Visual acuity 20/25 Enlarged scleral ring and thinned retinal pigment epithelium
Right eye	Visual acuity 20/400 PSCC	Visual acuity 20/400, degenerative myopia No cataracts
4. Cutaneous	Normal	Normal

* CNS = central nervous system; VS = vestibular schwannoma; PSCC = posterior subcapsular cataract; CAL = café-au-lait.

and 20/400 in the right eye. He had decreased hearing in his left ear 1 year prior to GE-MRI evaluation at age 13, which revealed bilateral VSs (left, dumbbell-shaped, 2.0 cm in diameter; right, 5.0 cm × 10.0 cm). There was enlargement and irregularity of the left optic nerve (possible optic nerve sheath meningioma), and enhancement of the left 4th and 9th–12th cranial nerves consistent with schwannomas. There were three cervical and thoracic spine tumors, including a 0.2–0.3 cm × 0.6–0.7 cm plaque-like lesion at C2-3, a 0.3 cm × 0.8–0.9 cm × 1.0 cm plaque-like lesion at T2, and a 0.8 cm × 1.2 cm lesion at T4-5.

At age 17 months, twin B had very high, degenerative myopia and ocular deviation out and down in the right eye. Visual acuity was 20/25 in the left eye and 20/400 in the right eye. Cataracts were not present. There was an enlarged scleral ring and thinned retinal pigment epithelium in the left eye. He was asymptomatic when he was imaged at age 13, after his twin was diagnosed. He had bilateral VSs and 3 nodules (the largest, 0.5 cm × 0.5 cm × 1.0 cm) at C3-4 and C5-6. There were no tumors in the thoracic or lumbar spines.

DISCUSSION

Given the overall clinical variability of NF2 [Evans et al., 1992a,b; Parry et al., 1994], there were striking similarities between these MZ twins. Each pair was concordant for general phenotypic subtype (pair 1, mild; pairs 2 and 3, severe) and often for the organ systems that were affected (Table I). Although the prevalence of clinical manifestations in NF2 increases with age [Parry et al., 1994], even the youngest twin pairs were phenotypically similar. There were, however, some differences in specific features of disease presentation or progression. For example, all three twin pairs were discordant for presence or type of associated in-

tracranial tumors. Early onset lenticular opacities and retinal abnormalities are associated with NF2 [Kaiser-Kupfer et al., 1989; Parry et al., 1994; Ragge et al., 1995], but some of the ocular abnormalities could be caused by other pathologic processes.

It is not surprising that some disease features differ *between* NF2 MZ twins, but certain potential causes can be eliminated. Disease phenotype can be affected by type of mutation [Merel et al., 1995], modifying genes, genomic imprinting [Kanter and Eldridge, 1978; Evans et al., 1992a], mosaicism [Bourn et al., 1992a], environment, stochastic processes (i.e., random processes operating over time), or interactions between these causes [Riccardi, 1993]. Type of mutation, modifying genes, genomic imprinting, and mosaicism cannot account for phenotypic differences between genetically identical twins with the same NF2 phenotypic subtype. There have been no studies of environmental influences on NF2, and it can be difficult to determine the environmental component of phenotype in MZ twins because their environment is usually more similar than for individuals in the general population. Stochastic processes that may cause phenotypic variability between NF2 MZ twins include the loss of the second *NF2* allele or alleles of other genes and their subsequent effects. We hypothesize, therefore, that the phenotypic differences between these NF2 MZ twins are most likely due to stochastic processes.

Phenotypic differences in NF2 MZ twins may be germane to NF2 genotype-phenotype correlations. Some NF2 patients cannot be clearly classified into either a mild or severe phenotype [Evans et al., 1992b; Parry et al., 1994]. It is possible that there is a molecular basis for the mild and severe phenotypes [Merel et al., 1995], but that variability introduced into phenotype by stochastic processes increases the difficulty of identifying genotype-phenotype correlations.

Genotype-phenotype relationships have been examined in patients with mutations in other tumor suppressor genes, including *NF1, RB1, VHL,* and *APC*. Genotype-phenotype correlations are not generally observed in NF1, although large *NF1* gene deletions may predispose patients to large numbers of neurofibromas for the patient's age [Kayes et al., 1994]. Mutations in exons 4, 16, and 20 in the *RB1* gene cause a mild phenotype and incomplete penetrance [Sakai et al., 1991; Onadim et al., 1992; Dryja et al., 1993; Lohmann et al., 1994]. *VHL* missense mutations are associated with a high frequency of pheochromocytoma [Chen et al., 1995].

Several genotype-phenotype correlations have been reported for the *APC* gene. Mutations in codons 463–1387 are associated with congenital hypertrophy of the retinal pigment epithelium (CHRPE) [Olschwang et al., 1993; Wallis et al., 1994]. Mutations in codons 1445–1578 are associated with severe desmoid tumors, but not with CHRPE [Caspari et al., 1995]. Mutations in the 5′ exons of the *APC* gene are correlated with a form of the disease that causes fewer colonic polyps and later onset of symptoms [Spirio et al., 1993]. Mutations between codons 1250 and 1464 are associated with larger numbers of colonic polyps [Nagase et al., 1993]. Deletions in codon 1309 cause severe disease, as manifested by earlier ages of onset of symptoms, diagnosis of polyps, and death [Caspari et al., 1994]. These correlations suggest that the length of the truncated *APC* gene product influences disease severity.

MZ twins have been reported in other tumor suppressor gene syndromes, including NF1 [Easton et al., 1993] and retinoblastoma [Liu, 1990]. In NF1, the correlation of CAL spots and neurofibromas is highest between MZ twins and decreases with decreasing degree of relation (a similar pattern occurs in binary traits), suggesting that NF1 phenotype is affected by modifying genes [Easton et al., 1993]. A similar quantitative analysis for NF2 is not possible at present, but may be in the future when additional twins are ascertained; in this regard the family of twin pair 1 would be of particular interest.

In summary, NF2 MZ twins were concordant for general NF2 phenotypic subtype, but some specific disease features were discordant. We hypothesize that these differences are most likely due to stochastic processes such as the loss of the second *NF2* allele or alleles of other genes, and their subsequent effects. Phenotypic differences between NF2 MZ twins suggest that mutational analysis may be limited in predicting specific features of NF2 presentation or progression, although mutational analysis still may be useful in predicting general phenotypic subtype. Moreover, DNA testing can lead to early diagnosis, which is essential for optimal management [CDP, 1994].

ACKNOWLEDGMENTS

We thank Karla Figueroa for zygosity analysis, Drs. Mia MacCollin, Dilys Parry, and Yaron Rabinowitz for manuscript review, Drs. Mark Borchert and Rena Falk for clinical examinations, and the NF2 patients and their families for participation. This work was supported in part by NIH L-08NSO1428-04, ACS VM-94, the Steven and Lottie Walker Foundation, the Carmen and Louis Warschaw Endowment Fund, House Ear Institute 484, the Texas NF Foundation, and the Vista Foundation.

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